Patent Number: GB2283913

Publication date: 1995-05-24

Inventor(s): AYUKO WASHINGTON ODUR

Applicant(s): RADOPATH LTD [GB]

Requested Patent: CN1140992

Application Number: GB19940021099 19941019

Priority Number (s): GB19930021558 19931019

IPC Classification: A61K31/525

Equivalents: AP620, AU7943794, BG100599, BR9407862, CA2123825, CA2174552, CZ9601137, EE9600057, EP0739208 (WO9511028), HR940688, HU76322, JP9505804T, MA23356, NO961547, OA10579, PL314008, SK50696, WO9511028, ZA9408191

Abstract

Flavins and their derivatives are useful for administration to mammalian subjects as an anti-viral agents, Riboflavin and riboflavin derivatives are particularly preferred for use in the treatment of HIV infection.

CLAIMS

CLAIMS

1. Use of a flavin, flavin derivative or a mixture comprising two or more thereof for the manufacture of a medicament for the treatment by prophylaxis or therapy of disease caused by viral infection.

2. Use as claimed in Claim 1 wherein the flavin derivative is riboflavin or a riboflavin derivative.

3. Use as claimed in Claim 2 wherein the riboflavin derivative is a riboflavin salt.

4. Use as claimed in Claim 3 wherein the riboflavin salt is riboflavin sodium phosphate or riboflavin tetrabutyrate.

5. Use as claimed in Claim 1 wherein the flavin or flavin derivative is a compound of the general

formula:

wherein R is hydrogen or alkyl;
R1 and R4 are, each independently, hydrogen, alkyl, hydroxy, halo, alkoxy, alkylthio, thio or an optionally substituted aromatic or non-aromatic nitrogen heterocycle, and X is: (i) hydrogen, ribityl, alkyl, hydrogen or an aromatic or non-aromatic carbocycle (ii) a group of the general formula: -CH2-(CHOH)n-Y in which n is an integer of 3 or 4 and Y is -CH2OH1-COOH or -COH or a group of the formula:

wherein R is hydrogen or alkyl; and wherein W1 and W2 are, each independently, alkyl, hydroxy, halo, alkoxy, alkylthio, thio or an optionally substituted aromatic or non-aromatic nitrogen heterocycle.

6. Use as claimed in Claim 1 wherein the flavin or flavin derivative is a compound of the general formula:

wherein X is (i) hydrogen, ribityl, alkyl, hydrogen or an aromatic or non-aromatic carbocycle (ii) a group of the general formula: CH2- (CHOH)I1 Y in which n is an integer of 3 or 4 and Y is - CH2OH1-COOH or -COH or a group of the formula:

wherein R is hydrogen or alkyl; and wherein W1 and W2 are, each independently, alkyl, hydroxy, halo, alkoxy, alkylthio, thio or an optionally substituted aromatic or non-aromatic nitrogen heterocycle.

7. Use as claimed in Claim 1 wherein the flavin or flavin derivative is a compound of the general formula:

wherein R1 is hydrogen or an alkyl group,
R2 is an alkyl group or a ribityl group, and
R3 represents hydrogen or mono- or di-substitution of the outer carbocyclic ring with an alkyl group.

8. Use as claimed in Claim 1, wherein the flavin or flavin derivative is lumichrome; roseflavin; a hydroxyflavin; an alloxazine or derivative thereof; an 8a-N(3)-histidylflavin; an 8a-N(1)-histidyl flavin; an 8acysteinyl thioether; an 6a-S-cysteinyl thioether; a lumiflavin; a 5-deazaflavin; a 5-carba-5-deaza or 1-carba l-deaza analog of riboflavin, FMN or FAD; flavin-1,N6 ethenoadenine dinucleotide; 9 -methylf lavin ; 9 -ph eny 1 f lav in; 9-benzylflavin; 9-cyclohexylflavin; 6, 9-dimethylflavin; 6,7,9-trimethylflavin; 9-oxyethylflavin; 9dioxypropylflavin; 6,8, 9-trimethylflavin; lacroflavin; flavin-9-carboxylic acid; 6, 7-dimethylflavin-9-carboxylic acid; or a schizaflavin.

9. Use as claimed in any preceding claim at a dosage regime of at least about 10 mg/kg of body weight per day.

10. Use as claimed in any preceding claim wherein the medicament is in injectable form.

11. A flavin or flavin derivative for use in the manufacture of a medicament useful in the treatment by prophylaxis or therapy of disease caused by viral infection.

12. A flavin or flavin derivative as claimed in Claim 11 and as defined in any one of Claims 2 to 8.

13. A pharmaceutical composition for the treatment by prophylaxis or therapy of disease caused by viral infection, the composition being characterized in that it comprises a flavin or flavin derivative.

14. A composition as claimed in Claim 13 wherein the flavin or flavin derivative is as defined in any one of
Claims 2 to 8.

15. A composition as claimed in Claim 12 or Claim 13 which composition comprises a unit dose of at least about 35 mg of a flavin or flavin derivative together with a pharmaceutically or veterinarily acceptable diluent, excipient or carrier.

16. A composition as claimed in Claim 15 wherein the unit dose is from about 35 mg to about 1000 mg.

17. A composition as claimed in Claim 16 wherein the unit dose is from about 250 to 500 mg.

18. A composition as claimed in any one of Claims 15 to 17 which is in injectable form.

19. A composition as claimed in Claim 18 in the form of a solution in sterile water.

20. A receptacle for pharmaceutical containment immediately pre-adminstration, said receptacle being manipulable in a drug adminstration procedure by medical practitioners and containing a flavin or flavin derivative for discharge from the receptacle to a patient or to an administration device and said receptacle carrying a representation of instructions for use of the flavin or flavin derivative as a medicament for the treatment by prophylaxis or therapy of disease caused by viral infection.

21. The combination of (a) a flavin or flavin derivative formulated for pharmaceutical use, and (b) instructions for use of said formulated flavin or flavin derivative for the manufacture of a medicament for the treatment by therapy or prophylaxis of disease caused by viral infection or for use thereof for said treatment.

22. The combination of Claim 21 wherein the treatment is referred to in the instructions and is the treatment of
HIV-infection.

23. The combination of Claim 22 wherein the HIV-infection is chronic infection.

24. A process for the manufacture of a medicament for use in the management and treatment of viral infection, which process comprises formulating a flavin or flavin derivative for anti-viral use.

25. Flavin, or a flavin derivative as an anti-viral agent, together with another compound(s) having anti-viral activity, as a combined preparation for simultaneous, separate or sequential use in anti-viral therapy.

26. A method for the treatment by prophylaxis or therapy of disease caused by viral infection which method comprises administering therapeutically to a patient suffering from such disease an effective amount of a flavin or a flavin derivative or administering prophylactically to a patient at risk of viral infection an effective amount thereof.

27. A method as claimed in Claim 26 wherein the amount administered is at least about 1 to about 10

or more mg/kg of patient body weight.

28. A flavin or a flavin derivative thereof not known for any pharmaceutical utility for use as an anti-viral agent.

29. A flavin or flavin derivative for use in the treatment by prophylaxis or therapy of a disease caused by viral infection.

30. A flavin or flavin derivative as claimed in Claim 9 and as defined in any one of Claims 1 to 8.

31. An anti-viral agent for use in the treatment of HIVinfection in a mammalian subject at least at a chronic infection stage, the agent having a cellular target and optionally also a viral target and being a flavin or flavin derivative acting intracellularly on cell metabolism in mammalian cells infected chronically or acutely with HIV to block or compensate for the effects of the viral infection on the cell in the asymptomatic and post-asymptomatic phases of the infection by the virus.

32. An anti-viral agent as claimed in Claim 31 and which is a riboflavin derivative.

33. A method of in vitro diagnostic assay which method comprises sampling the cells of a mammalian patient infected with HIV after treating the patient by a treatment regime in which a flavine or flavine derivative is administered to the patient, and performing an assay upon the cell sample externally of and separate from the patients body to determine the activity and/or progress of the viral infection.

DESCRIPTION

ANTI-VIRAL AGENTS

The present invention relates to anti-viral agents and their use in the treatment of human and animal patients to alleviate or cure the ill-effects caused by viral infection, especially HIV. A detailed study of compounds according to the invention has been carried out to evaluate their efficacy against infection from several strains of
HIV-1. The compounds have similar activity against HIV in both acutely and chronically infected cells. This is a dual property only ocassionally associated with other compounds which are in current use in the therapy of HIV infection although de nova (acute) infections of cells may be treated by compounds which act early in the replication cycle of HIV to block integration of vDNA into the host chromosome. It is this integration which signifies entry of the infection into the chronic state.Compounds which act post-integration of HIV are therefore inhibitors of chronically infected cells. Zidovudine (AZT) for example is only active against de nova infection of HIV and has no significant activity against chronically infected cells.

Inhibitors of gene expression of HIV (which is a positive strand RNA virus) would therefore be active in HIV chronically infected cells.

HIV is a positive strand RNA virus which affects humans.

The virus attaches to cell membranes by virion adsorption to CD4 surface receptor. The virion then passes through the cell membrane penetratively and enters the cell cytoplasm. Uncoating of the virion then takes place in the cytoplasm whereby the viral envelope and the protein coat of the genome

release the viral RNA in[redacted]e cytoplasm.

Reverse transcription therein produces a double-stranded
DNA transcript from host cell genetic material. This invades the host cell nucleus and integrates with the host cell chromosomal DNA. Transcription follows to produce a vRNA replicate which is translated in the cytoplasm to produce new viral proteins. The latter then assembles with vRNA at the inner cell surface to produce new virus particles which are released from the host cell.

HIV is normally associated with an initial asymptomatic phase. This initial asymptomatic phase may last a number of years before the early signs of HIV disease occur.

A number of ideas causing cell death are proposed.

Apoptosis is one of these. It is a morphologically distinctive form of programmed cell death involved in many physiological and pathological processes including cellular processes which seek to maintain appropriate intracellular oxidant-antioxidant balance. Cell death in T-cells is closely associated with this balancing process. Infection with HIV is thought gradually to disturb the balance in favour of cell death. Another critical factor in determining whether cells will grow and divide in a normal fashion is intracellular ATP concentration. Low intracellular levels of ATP are associated with ischemic death. T-lymphocytes are especially vulnerable to depletion of intracellular ATP levels. HIV infection may disturb cellular oxidative phosphorylation which is the cellular process responsible for ATP levels in the cell.

Cell death from whatever cause will eventually lead to cell depletion to a level that induces AIDS.

Much of the current work in the field of antiviral research is concerned with targeting specific viral encoded enzymes.

Compounds discovered from this research, in principle, should have low toxicity on cellular processes. The long term use of compounds in clinical trials in HIV infection treatment has not given the degree of benefit initially expected, and new approaches are needed.

Riboflavine is a known compound, which is also variously known as:
E101;
Lactoflavin;
Riboflavin;
Riboflavinum;
Vitamin B2;
Vitamin G;
7,8-Dimethyl-10-(1'-D-ribityl) isoalloxazine; and 3,10-Dihydro-7,8-dimethyl-10-(D-ribo-2,3,4,5-tetra-
hydroxypentyl) benzopteridine-2,4-dione.

Riboflavine is commercially available as such or as its sodium phosphate or tetrabutyrate salt, typically in the former instance as the dihydrate salt. It is also available in various mixtures with other vitamins, all essentially being for the treatment of, inter alia, vitamin
B deficiency. In such mixtures the dose of riboflavin varies between 0.5 and 10 mg, with a maximum recommended daily dose being 30 mg.

No adverse effects have been reported with the use of riboflavine. However, significant doses of

riboflavine result in a bright yellow discoloration of the urine which may interfere with certain laboratory tests.

The riboflavine requirement of humans is often related to the energy intake, but it appears to be more closely related to resting metabolic requirements. A daily dietary intake of about 1.3 to 1.8 mg of riboflavine is recommended that is to say the basic recommended intake of riboflavine is 550 ssg per 4200 kj (1000 kcal) of diet - Report of a
Joint FAO/WHO Expert Group, Tech. Rep. Ser. Wld 111th Org.

No. 362, 1967.

The estimated acceptable daily intake of riboflavine is up to 500 ssg per kg body weight - see Thirteenth Report of
FAO/WHO Expert Committee on Food Additives, Tech. Rep. Ser.

WHO. No. 445, 1971.

Riboflavine, which is a water-soluble vitamin, is essential for the utilisation of energy from food. The active, phosphorylated forms, flavine mono-nucleotide and flavine adenine dinucleotide, are involved as co-enzymes in oxidative/reductive metabolic reactions.

Various other flavins and derivatives thereof are also known, mainly as flavouring agents.

It has now been found surprisingly that the administration of riboflavine, as well as other flavins and derivatives thereof, at doses far higher than previously used or recommended can be highly effective in the management and treatment of viral infections, in particular HIV. The structure of the compound indicates involvement in the process of oxidative phosphorylation within cells. It is possible that the compounds of the invention preferentially target the same target as HIV and so resist or prevent the manifestations of infection including the procreative capacity of the virus.

Accordingly, the present invention in one aspect provides the use of a flavin, especially riboflavine, or a derivative thereof for the manufacture of a medicament for the management and treatment of viral infection.

Moreover, insofar as certain flavins and derivatives thereof are not known as pharmaceuticals, even in a general sense as with riboflavine (known as an enzyme co-factor vitamin), the invention in a second and broader aspect provides such certain flavins or a derivative thereof for use as anti-viral agents.

In the use according to the invention riboflavine or other flavin may be used as such or as a derivative and the flavin derivative may be any derivative which is safe for human or animal use. Preferably, however, in the case of riboflavine the derivative is a riboflavine salt and more preferably the riboflavine salt is riboflavine sodium phosphate or riboflavine tetrabutyrate. Most preferably, the flavin or derivative should be of high purity and contamination with spurious ingredients should be avoided.

In more general terms, the flavin or derivative for use in accordance with the invention may be defined as a compound of the formula (I), namely:

wherein:

(riboflavine-5'-phosphate sodium salt dihydrate)

(flavin-adenine dinucleotide) or CH3 (lumiflavin).

In addition, in the above formula (I) the group X may be alkyl, or H or an aromatic or other cyclic hydrocarbon group.

Thus, and furthermore, the use of the invention may be realised with flavins or derivatives such as:
(A) lumichrome of the formula:

(B) Roseoflavin of the formula:

(C) B-Hydroxyflavine, alloxazines and other derivatives thereof:

wherein
R is ribityl, alkyl, or H;
X is OH, Br, C1, -SH, OAlk or SAlk.

Some Examples of the above are:

R = alkyl
ribityl or rib-P (8-hydroxy-FMN)
R=Rib-P-AMP
(8-hydroxy-FAD)

wherein R is as above.

(D) 8a-N(3)-histidylflavins

wherein R denotes the ribityl side chain for the riboflavin derivative.

(E) 8aN(1)-histidylflavins:

wherein R denotes the ribityl side chain for the riboflavin derivative.

(F) 8-Cysteinylflavin thioethers: (G) 6-S-cysteinylflavin thioethers: (H) Lumiflavins:

wherein R1=R4=H, R2=R3=CH3 for lumiflavin itself.

(I) 5-Deazaflavins:
These may be illustrated by the following formula:

wherein the substituent groups are as defined below:
R1 R2 R3
H CH3 H
H C2H5 H
H n-C3H7 H
H n-C4Hg H

CH3 CH3 H
CH3 C2H5 H
CH3 n-C3H7 H
CH3 n-C4Hg H
H CH3 7,8-(CH3)2
H D-ribityl 7,8-(CH3)2
H C2H5 7 CH3
CH3 C2H5 7-CH3
CH3 D-ribityl 7,8-(CH3)3 and derivatives thereof such as:

(J) 5-Carba-5-deaza and l-carba-1-deaza analogs of
riboflavin, FMN, and FAD.

These may be illustrated by riboflavin analogs (X), 5carba-5-deazariboflavin analogs (XI) and l-carba-ldeazariboflavin analogs (XII), that is:

(K) Flavin 1, N6-Ethyenoadenine dinucleotide

(L) Schizoflavins and derivatives.

7,8-dimethyl isoalloxaz ine
Riboflavin 7,8-dimethylisoalloxazine
SF2 7,8-dimethyl
isolloxazine
SF1
The above are chemical structures of schizoflavins and show their formation from riboflavin. SF2 and SF1 can be identified as 7,8-dimethyl-10-(2,3,4-trihydroxy-4formylbutyl) isoalloxazine and 7,8-dimethyl-10-(2,3,4trihydroxy-4-carboxybutyl) isolloxazine, respectively.

Other flavins may be illustrated by:
3-carboxymethylriboflavin
3-carboxymethyl FMN 7-amino-10-(l'-D-ribityl) isoalloxazine
8-amino-7,10-dimethylisoalloxazine
8a(S-Mercaptopropionic acid) riboflavin
8a(S-Mercaptopropionic acid) FMN 8a(N-Aminohexyl)FMN
9-Azobenzoyl FMN 10-(X-carboxyalkyl)-7,8-dimethylisoalloxazine
In the use according to the invention the flavin such as riboflavin, or derivative thereof, is preferably employed at a high dose level significantly in excess of the doses currently used or recommended. Thus, typically the riboflavin or other flavin in the clinical trial is used in the present invention at a dosage regime of at least about 1 to about 100 or more (eg 10 or above) mg/kg of body weight per day.In addition, use according to the invention preferably is one wherein the medicament is in orally administrable form, especially as a capsule (eg twopart).

Additionally or alternatively the invention includes a pharmaceutical or veterinary composition for use in the management and treatment of viral infections and in unit dosage form, which composition comprises a unit dose of at least about 35 mg such as 50mg or more (eg 50 to 300 mg, such as 50 to

200 or 50 to 100mg) of [redacted]vin such as riboflavine or derivative the[redacted]of as described or defined herein, together with a pharmaceutically or veterinarily acceptable diluent, excipient or carrier.

A composition according to the invention is preferably one wherein the unit dose is from about 35 mg to about 1000 mg.

More preferably, the unit dose is from about 250 to 500 mg.

In addition, a composition according to the invention is preferably in oral or injectable form. Within that context a preferred composition is one as a solution in sterile water.

The invention also includes a process for the manufacture of a medicament for use in the management and treatment of viral infections, which process comprises formulating a flavin such as riboflavine, or a derivative such as the tetrabutyrate salt thereof for anti-viral use.

As will be appreciated, a process according to the above definition may be carried out using one or more of the additional features mentioned herein.

In addition, the invention includes a product containing a flavin such as riboflavine, or a derivative thereof, as an anti-viral agent, together with another compound(s) having anti-viral activity as a combined preparation for simultaneous, separate or sequential use in anti-viral therapy.

Again, a product according to the above definition may be one which includes one or more of the other specific features of the invention defined herein.

The invention further includes a method for the treatment of viral infection, which method comprises orally or parenterally administering an effective amount of a flavin such riboflavine, or a derivation thereof.

Preferably in a method according to the invention, the amount administered is 1 to 100 (eg at least 10) mg/kg of patient body weight.

Furthermore, the method is particulary useful when the virus is human immunodeficiency virus, HIV.

Once again, a method according to the invention may include one or more of the other specific features of the invention defined herein.

Most preferably, the invention is carried out with one or more of riboflavine, riboflavine sodium phosphate, flavinadenine dinucleotide, lumiflavin, lumichrome, or especially riboflavin tetrabutyrate, whose formula is set forth below: -

In Vitro Assay
The following in vitro assays were used to investigate the anti-viral activity against HIV of compounds in accordance with the invention:1 Acute Infection Assays
1.1 Standard Acute Assay
High titre virus stocks of the human immunodeficiency
virus HIV-1 (HTLV-lllB; were grown in H9 cells with
RPMI 1640 supplemented 10% fetal calf serum as growth medium. Cell debris was removed by low speed centrifugation and the supernatant stored at -700C until required.In a typical assay, C8166

Tlymphoblastoid cells w[redacted]incubated with 10TC1D50 HIV1 at 370[redacted]r 90 minutes and then washed three times with phosphate buffer saline (PBS). Aliquots of 2 x 105 cells were resuspended in 1.5ml of growth medium in 6ml culture tubes, and test compound at log dilutions from 0.2 to 200M was added immediately.

The test compound was dissolved in 70% ethanol and the final concentration of alcohol in the culture was < 1%.

Cultures were incubated at 370C for 72 hours in 5% CO2.

20041 of supernatant was taken from each culture and assayed by optical density measurement at 450nm for
HIV p24 core antigen (Kinchington et al 1989, Roberts et al 1990) using a commercial ELISA which recognises all the core proteins equally (Coulter Electronics
Ltd, Luton, UK). To determine the IC50 values standard curves were drawn from untreated cultures containing < 1% alcohol. AZT and ddC were used as internal controls. Assays were carried out in duplicate.

1.2 Deleted Medium Assay
In the standard assay system, cell cultures were harvested, split and fed with fresh medium approximately 18 to 24 hours before the start of
assay. Addition of fresh medium stimulates the cells
to enter a log phase of growth. To investigate the
effect of cells reaching confluence in conditions of
depleted media, cell cultures were fed and split at
72, 48 and 24 hours before being used in a standard
acute assay.

1.3 Light Exposure Assay
A freshly dissolved sample of test compound was split
into two aliquots. They were placed either in
daylight or the dark for two hours before being
subjected to standard acute assay.

1.4 Preincubation Assay
Target cells were preincubated with test compound at
log dilutions of 200 to 0.24M for 18/24 hours before
infection with HIV-1. Each sample concentration was
then treated individually as in the standard acute
assay.

2 Assays for Chronically Infected Cells
2.1 Standard Chronic Assay
H9 cells chronically infected with HIV-lrf (H9rf) were
washed three times with medium to remove extracellular virus and incubated with test compounds (200 to 0.2my) for three days. p24 antigen was then determined by optical density measurement at 450nm as described for the acute infection standard assay. To determine the
IC50 values standard curves were drawn from untreated cultures containing 1% alcohol. RO 31-8959 (Roche
Proteinase inhibitor) was used as an internal control.

Assays were carried out in duplicate.

2.2 Depleted Medium Assay
In the standard assay, cell cultures were harvested, split and fed with fresh medium approximately 18 to 24 hours before assay. Addition of fresh medium stimulates the cells to enter a log phase of growth.

To investigate the effect of cells reaching confluence in conditions of depleted media, cell cultures were fed and split at 72, 48 and 24 hours before being used in a standard acute assay.

2.3 Light Exposure Assay
A freshly dissolved sample of test compound was split into two aliquots. They were placed either in daylight or the dark for two hours before being subjected to standard chronic assay.

3 Toxicity Assay
To test for compound toxicity, aliquots of 2 x 105 uninfected cells were cultured with test compounds at the same log dilutions for 72 hours (1.1 and 2.1). The cells were then washed with medium and resuspended in 200cm1 of growth medium containing C14 protein hydrolysate. The cells were harvested after 5 or 20 hours and the C14 incorporation measured. Untreated cells were used as controls.

The assays were applied to the compounds identified in
Table 1 below:
Table 1
Code Compound F1 Riboflavine
5'phosphate
F2 Riboflavine
F3 Flavine adenine
dinucleotide
F4 Lumiflavin
F5 Lumichrome
F6 Riboflavin tetranicotinate
F7 Riboflavin tetrabutyrate
Initial assays were carried out in relation to the various compounds mentioned in Table 2 to achieve preliminary results. The IC50 results in Table 2 are subject to confirmation; they are not consistent with re-run assays conducted to date. Assay results are shown in the graphs forming the following drawings and in Tables 2 to 10 which follow:
Figure 1: Antigen as optical density (OD) for Compounds F2,
F4 (first antigen assay) and F5 at 450 nm versus
concentration (/iM). The dotted line at OD 0.371
represents IC50 (active).

Figure 2: Antigen optical density (OD) for Compounds F1 and
F3 at 450 nm versus concentration (cm). The
dotted line at OD 0.371 represents IC50 (active).

Figure 3: Toxicity as C14 uptake (dpm) versus concentration
(M) for Compounds F2, F3, F4 (first toxicity
assay) and F5. The dotted line at 6035 dpm

represents CC50 (non-toxic).

Figure 4: Toxicity as C14 uptake (dpm) versus concentration (M) for Compound F1. The dotted line at 6035 dpm represents CC50 (non-toxic).

Figure 5: Antigen optical density (OD) for Compound F4 (second antigen assay) at 450 nm versus concentration (cm). The dotted line at OD 0.371 represents IC50 (active).

Figure 6: Toxicity as C14 uptake (dpm) versus concentration (M) for Compound F4 (second toxicity assay).

The dotted line at 6035 dpm represents CC50 (non toxic).

Figure 7: Antigen optical density (OD) for Compounds F6 and F7 at 450 nm versus concentration (M). The dotted line at OD 0.371 represents IC50 (active).

Figure 8: Toxicity as C14 uptake (dpm) versus concentration (elm) for Compounds F6 and F7. The dotted line at 6035 dpm represents CC50 (non-toxic).

Figure 9: Antigen control (ddC)

As shown by the Tables, the test compounds were evaluated for activity against cells both acutely and chronically infected with HIV. Antiviral (IC50) and toxicity (CC50) data is shown below. In another series of experiments, compounds were tested in cell cultures in which fresh media was added at 72, 48 and 24 hours prior to infection. This experiment was carried out to investigate the effects of the compounds on cells in actively dividing and quiescent states. This data indicates that cells may be more sensitive to the test compounds when quiescent. The effect of light on stability, preincubation of target cells, and the activity against an African HIV-1 isolate were also investigated. Exposure to light for two hours had no effect on the activity of the compound.Preincubation with the target cells enhanced its activity and it showed significant activity against the Africa HIV-1 isolate.

Table 2 (Figures 1 to 4) - Acute Infection Standard Assay (1.1)
Compound No/ IC50 CC50 SI
Assay No (Figures 1 and 2) (Fiqures 3 and 4)
Fl/1 1 to 20 > 200
F1/2 400 > 1000 F1/3 0.1 (Figure 2) > 800 (Figure 4) > 8000
F2 3 (Figure 1) > 200 (Figure 3) > 60
F3 0.8 (Figure 2) > 200 (Figure 3) > 200
F4 1 (Figure 1) 150 (Figure 3) 150
F5 3 (Figure 1) > 200 (Figure 3) > 60
Table 3 (Figures 7 and 8) - Acute Infection Standard Assay (1.1)
Compound No/
Assay No IC50 CC50 SI
F7/1 27 (Figure 7) 130 (Figure 8) 5
F7/2 57 > 200 > 4

F7/3 10 70 7
F7/4 25 140 6
Table 4 - Chronic Infection Standard Assay (2.1)
Compound No/ Assav No IC CCso SI
F7/1 0.2 7 35
F7/2 > 20 > 20
F7/3 10 > 200 > 20
F7/4 4 75 19
F7/5 26 > 200 > 7
Table 5 - Acute Infection Depleted Medium Assay (1.2)
Compound 72 hours 48 hours 24 hours
No
IC50 CC50 IC50 CC50 IC50 CC50
F7 10 160 21 100 110 160
Table 6 - Chronic Infection Depleted Medium Assay (2.2)
Compound 72 hours 48 hours 24 hours
No
ICso CC50 IC50 CC50 IC50 CC50
F7 40 75 90 250 60 101
Table 7 - Acute Infection Light Radiation Exposure Assay
(1.3)
Compound No Daylight Darkness
IC50 CC50 IC50 CC50
F7 60 > 200 60 > 200
Table 8 - Acute Infection Preincubation Assay (1.4)
Preincubation of target cells with test compound for 24 hours before infection
Compound No IC50 CC50 SI
F7 5 120 24
Table 9 (Figures 5 to 8) - Acute Infection Standard Assay (1.1)
Compound No IC50 CC50 SI
F4 13 (Figure 5) 150 (Figure 6) 12
F6 30 - 60 (Figure 7) > 200 (Figure 8) min 3 -6
Table 10 - Acute Infection Standard Assay (1.1)
Assay applied to C8166 Cells (T-lymphoblastoid cells transformed and immortalized by HTLV) with an African HIV
Isolate (HIV-1 CBL4)
Compound No IC CCso SI
F7 4 150 37.5
Table 11 (Figures 10 to 12) - Acute Infection Standard
Assay (1.1)
Compound No IC50 CCso SI
F7 32 200 6.3 ddC (control) 0.2
The variation in the end points observed with Compound F7 may be due to the properties of the target lymphoblastoid cells.Even in synchronized cultures there may be subtle changes in the metabolic state of sub-populations of cells.

This is reflected in the shift in the end points observed in the paired antiviral and toxicity values from assay to assay (Table 3). The results tabulated in Tables 5 and 6 indicate that cell culture in active or quiescent states may have different sensitivities to the test compound.

Patient Treatment
Thirty-five patients were placed on therapy. Thirty had follow up medical visits.

i) General Condition of the Patients
Twenty patients out of thirty who came for follow-up visits reported an improvement in their general condition. The majority of these reported improvement insofar as malaise, appetite and weight gain was concerned. Two patients also reported improvement in skin rash with regression of skin lesions while one reported no new skin lesions developed while on therapy. One patient also reported improvement in impotence (which had been present for three months prior to onset of therapy), while two other patients reported cessation of long standing coryza.

ii) Sick Visits
Few patients attended clinic for unscheduled sick visits:1. One patient had recurrent abscesses as well as septic arthritis which persisted even on therapy.

2. Two patients had recurrent lower respiratory tract infections with one developing frank broncho-pneumonia during second week of therapy. Repeated smears for AAFBS have continued to be negative.

3. Two patients had pyrexia with no localizing signs and repeated blood smear for malarial parasites were negative and no significant growth on blood culture. One of these patients responded well to amoxycillin and is now afebrile.

4. One patient had gastroenteritis during the third week of therapy.

5. Oral and vulvo-vaginal candidiasis were reported by two patients, with the vulvo-vaginal candidiasis being recurrent as soon as a course of Nystatin pessaries and tablets was completed.

6. Two patients also reported recurrent attacks of herpes simplex genitalis.

iii) Toxicity
Most of the cases of tonicity reported occurred during the first two weeks of therapy and have been transient.

Two patients experienced pruritus which averaged four days during first week of therapy and cleared spontaneously without any supportive medication.

Four patients reported moderate diarrhoea during the first two weeks of therapy. This has averaged four days. This has been a difficult symptom to attribute as between it being a side effect or a natural manifestation of the HIV infection. However, the consistency of its appearance in the first week of therapy, and its transient nature makes it reasonable to suppose it is a side effect.

One patient reported drowsiness and another reported darkening of her urine. MSU was normal.

Two patients reported abdominal discomfort.

iv) Laboratorv Results
Three patients had transient rises in liver enzymes during the second to third week of therapy, with no clinical signs of liver disease. However, the enzyme levels always returned to normal.

The above clinic trial reports are the preliminary results of a clinical trial which has currently been in progress for several weeks using Compound F7 administered orally in capsule form (the capsules are as described in Example 4 below) dosage was:
Dose level 1: lmg/kg body weight per day orally in two divided dosages
Dose level 2: 2mg/kg body weight per day orally in two divided dosages
Dose level 3: lomg/kg body weight per day orally in two divided dosages
Dose level 4: 1Smg/kg body weight per day orally in two divided dosages
Dose level 5: 20mg/kg body weight per day orally in two divided dosages
Dose level 6: 30mg/kg body weight per day orally in two to three divided dosages
Dose level 7: 40mg/kg body weight per day orally in two to three divided dosages
Dose level 8: 50mg/kg body weight per day orally in two to three divided dosages
Dose level 9: 100mg/kg body weight per day orally in two to three divided dosages
The following specific Examples illustrate compositions formulated in accordance with the invention:
Example 1
A formulation can be prepared from the following:
riboflavine-5-phosphate 10 mg
sterile water 2 ml to provide a unit dosage of 10 mg of riboflavine for administration once per day in the treatment of viral infection.

ExamPle 2
A formulation can be prepared from the following:
riboflavine-5-phosphate 30 mg
sterile water 2 ml to provide a unit dosage of 30 mg of riboflavine for administration once per day in the treatment of viral infection.

Example 3
Similar formulations to those of Examples 1 and 2 can be prepared at doses of:
10 mg per ml,
25 mg per ml, and
50 mg per ml respectively, in either a unit amount of 2 ml or 5 ml of sterile water and based on an active ingredient which is:
Riboflavine 5'phosphate Ribof lavine
Flavine adenine dinucleotide
Lumiflavin
Lumichrome or a mixture thereof.

Example 4
The following capsules were formulated:
Sizes: 25mg
50mg 100mg

200mg
400mg
Type: 2-part hard gelatin
Composition: Compound F7 in admixture with
microcrystalline cellulose Ph. Eur 166.4/156.7/118.6/108.7/50mg to give capsule weights of
191.4/206.7/218.6/ 308.7/450mg.

123: 65834c **ultramolecular structure-type drug delivery system for cytokines.** Sunamoto, Junzo; Akyoshi, Kazunari; Iwasa, Susumu; Sato, Jun (Takeda Chemical Industries Ltd) **Jpn. Kokai Tokkyo Koho JP 07 97,333 [95 97,333]** (Cl. A61K38/00), 11 Apr 1995, JP Appl. 93/193,644, 04 Aug 1993; 14 pp. Ultramol. structure-type drug delivery system for cytokines (e.g. interleukin-2) is prepd. by embedding cytokines to be delivered in polysaccharide-sterol derivs. (e.g. pullulan-cholesterol deriv.: prepn. given). Capsules were formulated contg. the interleukin-2-embedded structure 10, lactose 90, microcryst. cellulose 70, and magnesium stearate 10 mg. Bioavailability was excellent after administration.

123: 65835d **Pharmaceutical compositions containing flavin derivatives as anti-viral agents.** Washington, Odur Ayuko (Radopath Ltd.) **PCT Int. Appl. WO 95 11,028** (Cl. A61K31/525), 27 Apr 1995, GB Appl. 93/21,558, 19 Oct 1993; 52 pp. Pharmaceutical compns. contg. various flavin derivs. (Markush structure given) are disclosed for administration to mammalian subjects as an anti-viral agent. The efficacy of 1-100 mg/day oral riboflavin tetrabutyrate (I) in treatment of patients who had viral infections is reported. A capsule contained I 25, and microcryst. cellulose 166.4 mg.

123: 65836e **Pharmaceutical unit dosage form containing picosulfate.** Mandel, Kenneth Gary; Davis, Paula Denise (Procter and Gamble Co.) **PCT Int. Appl. WO 95 11,024** (Cl. A61K31/44), 27 Apr 1995, US Appl. 139,364, 19 Oct 1993; 18 pp. A pharmaceutical laxative compn. in unit dosage form, for peroral administration of picosulfate (I) to a human or other animal subject, comprises a safe and effective amt. of I in a rapidly dissolving matrix; and a proximal colonic delivery carrier which effects release of I substantially near the junction between the small intestine and the colon or within the colon of the subject. I 5.0, was blended with dextrates 25.0, and lactose 0.2 mg/unit dose and filled into a hard gelatin capsule. Eudragit S-100 8.7, di-Bu phthalate 1.7, talc 2.3, and Fe_2O_3 1.3 mg/unit dose were mixed in iso-Pr alc. and the resulting suspension was applied to the hard gelatin capsule.

123: 65837f **Treatment of urinary incontinence and other disorders with (R)-terodiline and hydroxylated derivatives thereof.** Gray, Nancy M.; Young, James W. (Sepracor, Inc.) **PCT Int. Appl. WO 95 10,269** (Cl. A61K31/135), 20 Apr 1995, US Appl. 134,271, 08 Oct 1993; 29 pp. Methods and compns. are disclosed utilizing the optically pure R-isomers of terodiline or of the hydroxylated derivs. of terodiline. These compds. are potent drugs for the treatment of urinary incontinence, obstructive pulmonary disease and such other conditions as are related to the compds.' activity as anticholinergic agents. Use of these compds. substantially reduces adverse effects assocd. with the administration of racemic terodiline. The compds. are administered by i.v. infusion, transdermal patches, tablets, capsules, or inhalants.

123: 65838g **Liposomes encapsulating doxorubicin and anti-free radical agents.** Pons Lambiez, Fernando; Delgado Gonzalez, Raquel; Parente Duena, Antonio (Lipotec, S.A.) **Eur. Pat. Appl. EP 655,239** (Cl. A61K9/127), 31 May 1995, ES Appl. 9,302,481, 25 Nov 1993; 20 pp. Antitumor liposomes comprise doxorubicin and benzopyrans to decrease the toxicity of the drug when it is administered in i.v. form. For example, a liposome contg. a mixt. of hydrogenated phospholipids, phosphatidylglycerols, cholesterol, and doxorubicin at the wt. ratio of 50:50:50:15 and 7.5 mg 6,7-dimethoxy-2,2-dimethyl-2H-chromene was prepd. and i.p. injected to rats to demonstrate no mortality; in contrast, when the same doses of doxorubicin were i.p. inoculated in the form of free drug, a 100% mortality was obsd.

123: 65839h **Taxol formulation for cancer treatment.** Straubinger, Robert M.; Sharma, Amarnath; Mayhew, Eric (New York State University) **U.S. US 5,415,869** (Cl. 424-450; A61K9/127), 16 May 1995, Appl. 151,215, 12 Nov 1993; 28 pp. A liposome formulation contg. a taxane for cancer treatment contain a mixt. of one or more neg. charged phospholipids and one or more zwitterion (i.e. uncharged) phospholipids in ratio 1:9-7:3, resp. The taxol liposomes were prepd. by hydration of a lyophilized powder contg. drug and phospholipids and their toxicity and cytostatic and antitumor activities were evaluated.

123: 65840b **Compositions of gastric acid-resistant microspheres containing salts of bile acids.** Sipos, Tibor (Digestive Care Inc.) **U.S. US 5,415,872** (Cl. 424-490; A61K9/16), 16 May 1995, US Appl. 902,578, 22 Jun 1992; 10 pp. Cont.-in-part of U.S.5,352, 460. A bile salt compn. for treatment of bile salt deficiency contain a bile salt, a buffering agent, a disintegrant, an adhesive polymer, and a non-porous gastric acid-resistant polymer coating. Microspheres contg. Na ursodeoxycholate 68.1, disintegrant 4.3, anhyd. buffering agent 11.2, adhesive polymer 2.6, and a polymer coat-talc mixt. 13.8% by wt., resp., were formulated.

123: 65841c **Benzopyrans for treating dermatitis and related conditions.** Labroo, Virender M.; Piggott, James R. (ZymoGenetics, Inc.) **U.S. US 5,416,098** (Cl. 514-320; A61K31/395), 16 May 1995, Appl. 175,840, 30 Dec 1993; 7 pp. Oral and topical pharmaceutical compns. for treatment of dermatitis and conditions characterized by hyperproliferation of keratinocytes, such as psoriasis, contain 2,3-diaryl-1-benzopyrans and their salts. 2-[4-(2-Piperidinoethoxy)phenyl]-3-phenyl-2H-1-benzopyran 100 mg was mixed with 10 mL of colloidal solvent contg. 3 parts by vol. of di-Et ether and one part by vol. EtOH to obtain a topical soln. for direct application to affected skin using a dropper.

123: 65842d **Slimming compositions containing inhibitors of adipocyte glucose uptake.** Soudant, Etienne; Nadaud, Jean-Francois (Oreal S. A.) **Eur. Pat. Appl. EP 655,235** (Cl. A61K7/48), 31 May 1995, FR Appl. 93/14,156, 26 Nov 1993; 11 pp. A slimming compn. contains inhibitors of glucose uptake by adipocytes o lipolysis stimulant. A capsule contained Aerosil-200 5, Zn stearat talc 5, serin 200, and lactose q.s. 200 mg.

123: 65843e **Melatonin derivatives for use in treating sl disorders.** Flaugh, Michael Edward (Lilly, Eli, and Co.) **Eur. P Appl. EP 655,243** (Cl. A61K31/40), 31 May 1995, US A 154,332, 18 Nov 1993; 10 pp. The present invention provids method of treating sleep disorders using various melatonin anal A capsule was formulated contg. (±)-N-[2-methyl-2-(5-methoxy 6-chloroindol-3-yl)ethyl]acetamide 50, dried starch 200, and stearate 10 mg.

123: 65844f **Adhesive transdermal preparations containi 3-ketodesogestrel and optional 17-β-estradiol.** Kuroda, Hiro (Sekisui Chemical Co Ltd) **Jpn. Kokai Tokkyo Koho JP 07,101,** [95,101,864] (Cl. A61K31/565), 18 Apr 1995, Appl. 93/246,750, Oct 1993; 12 pp. The title prepns. comprise a support havi thereon an adhesive layer contg. adhesives 100, tackifiers 10-45, drug selected from 3-ketodesogestrel (I) and its 17-esters 3- transdermal absorbefacients 3-5, and releasing promoters for t absorbefacients 3-7 parts and the adhesives are acrylic copolym composed of ≥2 monomers selected from 2-ethyhexyl (meth)acryl and dodecyl methacrylate, the absorbefacients are amides of C aliph. monocarboxylic acids with monoethanolamide or diethanolamid and the releasing promoters are esters of C_{10-18} fatty acids with C alcs. The prepns. addnl. contain 3-7 parts ≥1 drug selected fro 17-β-estradiol (II) and its esters. I is useful as an contraceptive a amelioration of climacteric disorders and II is useful as amelioratio of climacteric disorders, osteoporosis, and menstral disorder Dodecyl methacrylate-2-ethylhexyl acrylate-2-ethylhexyl methacry late-hexamethylene glycol dimethacrylate copolymer (adhesive) 10 Ester Gum 20, I 4, lauric acid diethanolamide 4, and iso-I myristate 6 parts were mixed and dild. with AcOEt to give 500 pa adhesive compn. contg. 28 wt.% solid. A silicone-coated PET fil was coated with the adhesive compn. and the adhesive layer w transferred onto a PET-ethylene-vinyl acetate copolymer lamina film to give a transdermal prepn. The prepn. showed good adhesio over 24 h and caused no erythema.

123: 65845g **Sustained-release preparations containing poly-γ-(D-glutamic acid).** Sumya, Tooru; Sakurai, Juji (Kanegafuch Chemical Ind) **Jpn. Kokai Tokkyo Koho JP 07,101,880 [95,101,880** (Cl. A61K47/34), 18 Apr 1995, Appl. 93/245,018, 30 Sep 1993; pp. Sustained-release prepns. contg. the title compd. (I) as a bas material conferring sustained releasability. A mixt. of I an methylene blue (40:60) was kneaded with a small amt. of H_2O an dried to give a cube (1.5 × 1.5 mm). The cube was incubated with filtrates, obtained by suspending gastrointestinal contents (smal intestine, cecum, and large intestine) of mice in isotonic phosphat buffer soln. and filtering, at 37° under shaking. Releasing rate o methylene blue from the cube into the ext. from small intestine wa 7.9, 16.0, 25.0, 29.2, and 72.0% after 1, 2, 4, 6, and 22 h, resp., vs. 5.2 8.5, 10.6, 16.1, and 20.7%, resp., for a control by incubation wit isotonic phosphate buffer soln. Sustained-release prepns. cotng kanamycin sulfate, vitamin B_2, indomethacin, egg white lysozyme etc. were also prepd.

123: 65846h **Biodegradable polymer microspheres for controlled release of drugs or hormones.** Modi, Pankaj **U.S. US 5,417,982** (Cl. 424-486; A61K9/14), 23 May 1995, Appl. 197,756, 17 Feb 1994; 5 pp. A controlled release formulation for use with a variety of drugs or hormones are formed in microspherical form. The drug or hormone, e.g. bovine somatotropin, is suspended in a polymer matrix. The polymer matrix is formed from at least two highly water sol. biodegradable polymers, selected for example from starch, crosslinked starch, ficoll, polysucrose, polyvinyl alc., gelatin, hydroxymethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl Et cellulose, hydroxypropyl Me cellulose, sodium CM-cellulose, cellulose acetate, sodium alginate, polymaleic anhydride esters, polyortho esters, polyethyleneimine, polyethylene glycol, methoxypolyethylene glycol, ethoxypolyethylene glycol, polyethylene oxide, 1,3 bis(p-carboxyphenoxy) propane-sebacic anhydride copolymer, N,N-diethylaminoacetate, block copolymers of polyoxyethylene and polyoxypropylene. The microspheres are coated with a DL-lactide-glycolide copolymer. The coating makes the microspheres more resistant to enzymic degrdn.

123: 65847j **Pharmaceutical compositions containing tannic acid for the treatment of skin diseases.** Clodman, Percy B.; Clodman, Ossie **Can. Pat. Appl. CA 2,126,704** (Cl. A61K31/70), 08 Jan 1995, US Appl. 86,994, 07 Jul 1993; 19 pp. A dermatol. pharmaceutical compn. for stimulating hair growth and for the treatment of conditions of the skin selected from the group consisting of (a) benign moles, papilomas and seborrheas neratoses; (b) unsightly freckles, pimples and blemishes; (c) stasis dermatitis; (d) dermal ulcers; and (e) fungal nail infections; and (f) gingival and mucous membrans inflammations; the compn. comprising an effective amt. of tannic acid, a debriding agent and a pharmaceutically acceptable carrier therefore. A soln. contained anhyd. dextrose 10.00, liq. glucose 15.00, tannic acid 2.00, aloe powder 0.75, Me paraben 0.10, Pr paraben 0.05, iso-Pr alc. 2.00, zinc sulfate 0.10, glycerin 42.00, propylene glycol 20.00, and water 8.00%. The efficacy of the compn. in the treatemnt of skin disease in 2 patients is reported.

123: 65848k **Sustained-release preparation of anti-endothelin substance.** Igari, Yasutaka; Ikeda, Hitoshi; Tsuda, Masao; Yamamoto, Kazumichi; Wakimasu, Mitsuhiro (Takeda Chemical Industries, Ltd.) **Can. Pat. Appl. CA 2,126,619** (Cl. C07K7/64), 25 Dec 1994, JP Appl. 93/153,393, 24 Jun 1993; 63 pp. A sustained-release prepn. for treatment of endothelin-assocd. diseases contains an endothelin antagonist (a peptide) and a biodegradable

[19]中华人民共和国专利局

[51]Int.Cl⁶
A61K 31/525
A61K 31/505 A61K 31/70

[12] 发明专利申请公开说明书

[21] 申请号 94194350.X

[43]公开日 1997 年 1 月 22 日

[11] 公开号 CN 1140992A

[22]申请日 94.10.19
[30]优先权
[32]93.10.19[33]GB[31]9321558.0
[86]国际申请 PCT/GB94/02292 94.10.19
[87]国际公布 WO95/11028 英 95.4.27
[85]进入国家阶段日期 96.5.31
[71]申请人 拉多帕思有限公司
地址 英国泽西岛
[72]发明人 O·A·华盛顿

[74]专利代理机构 中国国际贸易促进委员会专利商标事务所
代理人 李 琰

权利要求书 7 页 说明书 27 页 附图页数 5 页

[54]发明名称 用作抗病毒剂的黄素衍生物

[57]摘要

公开了作为抗病毒剂用于哺乳动物的各种黄素衍生物。给出了可作为优选的特定实例的核黄素及核黄素衍生物。

(BJ)第 1456 号

说　明　书

用作抗病毒剂的黄素衍生物

本发明涉及抗病毒剂和它们在治疗人和动物患者，以及缓解或治疗由病毒特别是 HIV 感染引起的疾病中的应用。为估计它们对抗几种 HIV－1 病毒株感染的效力，已深入研究了本发明的化合物。这些化合物在对抗急性和慢性感染之细胞中的 HIV 方面具有相似的活性。这是一种仅仅偶而与目前用于治疗 HIV 感染的其他化合物相关联的双重特性，但可用早期作用于 HIV 复制周期的阻止 vDNA 整合到宿主染色体内的化合物治疗细胞的重新（急性）感染。正是这种整合作用指明感染进入慢性阶段。因此在 HIV 整合后发挥作用的化合物即为受慢性感染之细胞的抑制剂。例如齐多夫定（Zidovudine,AZT）只有抗 HIV 急性感染的活性而没有明显的抗慢性细胞的活性。因此 HIV（其为正链 RNA 病毒）之基因表达的抑制剂应是在 HIV 慢性感染的细胞中有活性的。

HIV 是感染人的正链 RNA 病毒。病毒颗粒吸附到 CD4 表面受上后病毒即附着于细胞膜上。然后病毒颗粒穿透通过细胞膜并进入细胞胞质中。然后病毒颗粒在胞质中脱包被，从而病毒被膜和基因组的蛋白质外被将病毒 RNA 释放到胞质中。在其中从宿主细胞遗传材料逆转录产生双链 DNA 转录特。该转录物侵入宿主细胞核并与宿主细胞染色体 DNA 整合。转录后产生 vRNA 复制品，进而在胞质中被翻译以产生新的病毒蛋白。然后病毒蛋白质在细胞内表面上与

vRNA 组装以产生可从宿主细胞中释放出的新的病毒颗粒.

HIV 正常情况下是与初始的无症状期相联系的. 在出现早期 HIV 疾病征象之前, 这种初始无症状期可持续数年.

已提出了许多引起细胞死亡的观点. 编程性细胞死亡即是其中之一. 其为一种涉及许多生理学和病理学过程的不同形成的编性细胞死亡, 其中力图保持适当的细胞内氧化剂一抗氧化剂平衡的细胞过程. T 细胞的细胞死亡是与这种平衡过程密切关联的. 认为 HIV 感染逆扰乱有利于细胞死亡的平衡. 确定细胞是否将以正常方式生长和分裂的另一个关键因素是细胞内 ATP 浓度. 低细胞内 ATP 水平与局部缺血性死亡有关. T 淋巴细胞对耗减细胞内 ATP 水平是特别易受损害的. HIV 感染可干扰细胞氧化磷酸化这一对细胞内 ATP 水平负责的细胞过程. 不管细胞死亡原因如何最终都将导致细胞减少到诱发艾滋病（ ADIS ）的水平.

在抗病毒研究领域, 目前大量的工作是涉及及靶向特异性病毒编码的酶. 之一研究发现的化合物原则上都应是对细胞过程低毒性的. 在治疗 HIV 感染的临床试用中长期使用这些化合物并没有达到原来预期的效果, 而需要进行新的研究.

核黄素是有下列不同名称的已知化合物:

E101 ;

乳黄素;

核黄素（ Riboflavin,Riboflavium ）;

维生素 B2 ;

维生素 G ;

7, 8 - 三甲基 - 10 - （ 1'- D - 核糖醇基）异咯嗪; 和

3，10－二氢－7，8－二甲基－10－（D－核糖－2，3，4，5－四羟戊基）苯并喋啶－2，4－二酮.

核黄素可作为基本身或其磷酸钠或四丁酸盐，特别是作为前者的二水合物盐从市场上购得．也可以作为与其他维生素的各种混合物得到之，所有这些产品基本上都是用于治疗维生素B缺乏．在这样的混合物中，核黄素的剂量在0.5和10mg之间，最大推荐用量为每天30mg．

尚未报导过使用核黄素引起的不良作用．但大剂量核黄素可使尿呈亮黄色从而干扰某些实验室检验．

人的核黄素需求常常与能量摄入有关，但似乎与静止代谢需要量关系及密切．推荐每天食物中核黄素的摄入量约为1.3至1.8mg，即是说推荐的核黄素基本摄入量为每4200kj(1000Kcal）食物500mg(参见Report of a Joint FAO/WHO Expert Group,Tech.Rep.Ser.Wld 111th Org.No.362,1967）．

估计的可接受的核黄素每天摄入量高达每kg体重500mg（参见Thirteenth Report of FAO/WHO Expert Committee on Food Additives,Tech.Rep.Ser.WHO.No.445,1971）．

作为水溶性维生素的核黄素，其对于食品能量的利用是必不可分的．活性的，磷酸化形式的核黄素，即黄素单核苷酸和黄素腺嘌呤二核苷酸作为辅酶参于氧化/还原代谢反应．

各种其他黄素和其衍生物也是已和的，并主要是用作调味剂．

现已令人惊奇地发现，以远比从前使用的或推荐的剂量更高剂量投用核黄素及其他黄素和其衍生物，可以十分有效地控制和治疗病毒特别是HIV感染．该化合物结构表明其参于细胞内的氧化磷酸

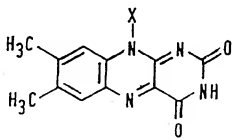
化过程．本发明的化合物有可能优先击中如 HIV 的同样靶目标，从而对抗或防止出现包括病毒之产生能力在内的感染征象．

因此，本发明的一个方面是涉及使用黄素，特别是核黄素或其衍生物制造用于控制和治疗病毒感染的药物．

再者，就尚不知道某些黄素其衍生物可以作为药物来说－甚至在一般意义上的核黄素也是这样（也知为一种酶辅助因子维生素），所以本发明在第二个和更宽的方面涉及这些黄素或其衍生物作为抗病毒剂的应用．

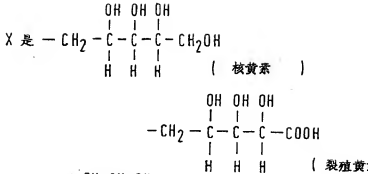
在按照本发明的应用中，可以作为本身或衍生物来使用，核黄素或其他黄素，并且黄素衍生物可以是对人或动物使用安全的任何衍生物．但在使用核黄素的情况下，衍生物较好是核黄素盐，更好是核黄素磷酸钠或核黄素四丁酸盐．黄素或其衍生物最好是高纯度的，并且避免假成分的污染．

一般说来，用于本发明的黄素或衍生物可限定为式（Ⅰ）的化合物，即：



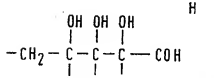
(I)

其中：

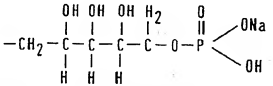


（核黄素　　）

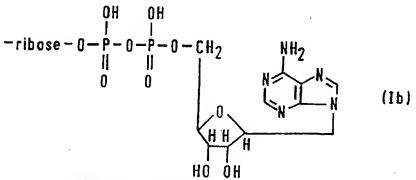
（裂殖黄素　　SF_1）



（裂殖黄素　　SF_2）



（核黄素－5′－磷酸钠盐二水合物）



(Ib)

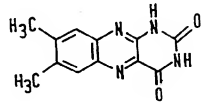
（黄素腺嘌呤二核苷酸）

或 CH_3（光黄素）

另外，在如上式（I）中基团 X 可以是烷基，或 H 或芳香基团或其他环烃基团。

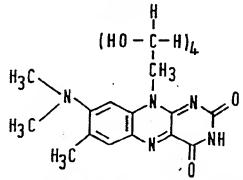
因此，可用黄素或下示衍生物实现本发明的应用：

（A）下式的光色素：



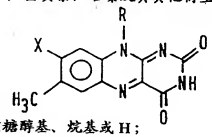
(II)

（B）下式的玫瑰黄素：



(III)

（C）B－羟基黄素、咯嗪及其其他衍生物：

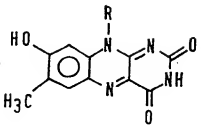


(IV)

其中 R 是核糖醇基、烷基或 H；

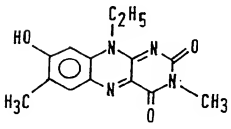
X 是 OH、Br、Cl、－SH、OAlk 或 SAlk。

上述化合物的某些例子是:

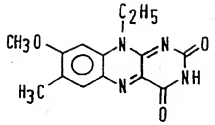


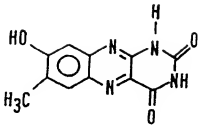
R = 烷基
核糖

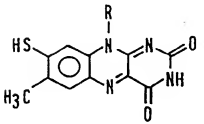
或 rib-P
(8- 羟基 -FMN)



R=Rib-P-AMP
(8- 羟基 -FAD)

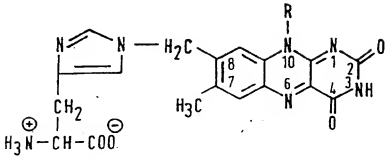






其中R的定义同上。

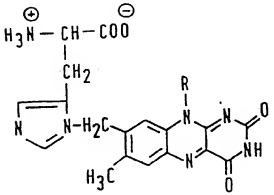
（D）8α－N（3）－组氨酰黄素：



(V)

其中R代表核黄素衍生物的核糖醇基侧链。

（E）8αN（1）－组氨酰黄素：



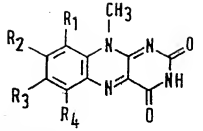
(VI)

其中R代表核黄素衍生物的核糖醇基侧链。

（F）8α－半胱氨酰黄素硫酯：

（G）6－S－半胱氨酰黄素硫酯：

（H）光黄素：

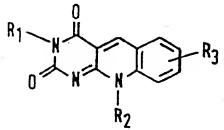


(VII)

其中光黄素本身的 $R_1 = R_4 = H$，$R_2 = R_3 = CH_3$。

（Ⅰ）5－脱氮杂黄素：

这类化合物的例子是：



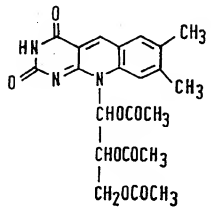
(VIII)

其中取代基限定如下：

R^1	R^2	R^3
H	CH_3	H
H	C_2H_5	H
H	n-C_3H_7	H
H	n-C_4H_9	H
CH_3	CH_3	H
CH_3	C_2H_5	H
CH_3	n-C_3H_7	H
CH_3	n-C_4H_9	H

H	CH_3	7,8-$(CH_3)_2$
H	D-核糖醇基	7,8-$(CH_3)_2$
H	C_2H_5	7 CH_3
CH_3	C_2H_5	7-CH_3
CH_3	D-核糖醇基	7,8-$(CH_3)_3$

且其衍生物例如有:



(IX)

(J)核黄素、FMN和FAD的5-碳酰-5-脱氮杂和1-碳酰-1-脱氮杂类似物.

这类化合物的例子是核黄素类似物(X)、5-碳酰-5-脱氮杂核黄素类似物(XI)和1-碳酰-1-脱氮杂核黄素类似物,即:

权 利 要 求 书

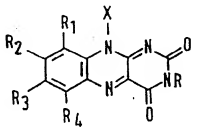
1.应用黄素、黄素衍生物或包括其中两种或多种成分的混合物制造用于预防或治疗由病毒感染引起的疾病的所述药物。

2.权利要求 1 所述的应用，其中黄素衍生物是核黄素或核黄素衍生物。

3. 权利要求 2 所述的应用，其中核黄素衍生物是核黄素盐。

4. 权利要求 3 所述的应用，其中核黄素盐是核黄素磷酸钠或核黄素四丁酸盐。

5. 权利要求 1 所述的应用，其中黄素或黄素衍生物是有下列结构通式的化合物：



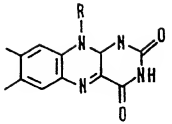
(VIIa)

其中 R 是氢或烷基；

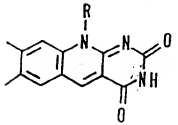
R_1 和 R_4 各自是氢、烷基、羟基、卤素、烷氧基、烷基硫代、硫代或可被取代的芳族或非芳族氮杂环，且 X 是：

（ i ）氢、核糖醇基、烷基、氢或芳族或非芳族碳环；

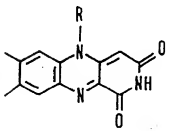
（ ii ）下列通式的基团：



(X)

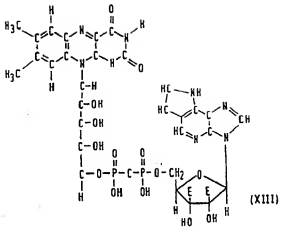


(XI)



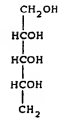
(XII)

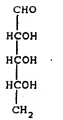
（K）黄素1，N^6－亚乙烯腺嘌呤二核苷酸

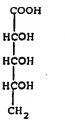


(XIII)

（L）裂殖黄素和衍生物：







7,8 －二甲基－异咯嗪　7,8 －二甲基－异咯嗪　7,8 －二甲基异咯嗪

核黄素　　SF2　　SF1

如上所示的是裂殖黄素的化学结构，并显示它们从核黄素的生成. SF2 和 SF1 可以分别鉴定为 7， 8 －二甲基－ 10 －（ 2， 3， 4 －三羟基－ 4 －甲酰丁基)异咯嗪和 7， 8 －二甲基－ 10 －（ 2， 3， 4 －三羟基－ 4 －羟丁基）异咯嗪.

可以作为举例的其他黄素有:

3 －羧甲基核黄素

3 －羧甲基 FMN

7 －氨基－ 10 －（ 1'－ D －核醇基）异咯嗪

8 －氨基－ 7， 10 －二甲基异咯嗪

8 α（ S －巯基丙酸）核黄素

8 α（ S －巯基丙酸） FMN

8 α（ N －氨乙基） FMN

9 －偶氮苯甲酰基 FMN

10 －（ W －羧基烷基）－ 7， 8 －二甲基异咯嗪

在根据本发明的应用中，较好以比目前使用或推荐的剂量明显

高的剂量水平使用黄素如核黄素或其衍生物．在本发明中，以至少每天每公斤体重大约 1 至 100mg 或更多（例如 10mg 或更多）的剂量使用核黄素或其他临床试用的黄素．另外，按照本发明的应用较好是作为口服给药剂型，特别是胶囊剂（如两部分的胶囊）用药．

本发明还包括用于控制和治疗病毒感染的和单位剂量形式的医药和兽药组合物，该组合物包括至少约 35mg 如 50mg 或更多（如 50 至 300mg， 50 至 200mg 或 50 至 100mg ）的单位剂量的本文所描述或限定的黄素如核黄素或其他衍生物，连同医药或兽药上可接受的稀释剂、赋形剂或载体．

根据本发明的组合物较好是其中单位剂量为大约 35mg 至 1000mg，更好是大约 250mg 至 500mg．

此外，根据本发明的组合物优选为口服剂型或注射剂型．其中优选的组合物是以无菌水溶液的形式存在．

发明也包括制备用于控制和治疗病毒感染的药物的方法，该方法包括配制黄素如核黄素或其衍生物如四丁酸盐用于抗病毒．

应认识到，根据以上定义的方法可以用本文所述的一个或多个附加的特征来实现．

另外，本发明包括一种用于抗病毒治疗的同时、分别或相继给药的结合制剂，其中含有作为抗病毒剂的黄素如核黄素或其衍生物，连同另一种具有抗病毒活性的化合物．

另外，根据上述定义的产品可以是包括本文限定之本发明的一个或多个其他具体特征的产品．

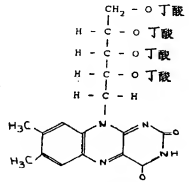
本发明进一步包括治疗病毒感染的方法，该方法包括口服或胃肠道外给予有效量的黄素如核黄素或其衍生物．

在本发明的方法中，给药量较好为每公斤患者体重 1 至 100（如至少 10 ） mg .

再者，当病毒是人免疫缺陷病毒 HIV 时，该方法特别有用.

还有，本发明的方法可包括一个或多个本文限定的本发明的其他具体特征.

最好用一种或多种核黄素、核黄素磷酸钠、黄素腺嘌呤二核苷酸、光色素、光黄素，或特别是有下文结构式的核黄素四丁酸完成本发明:



体外试验

使用下述体外试验法研究本发明的化合物抗 HIV 的抗病毒活性:

1.急性感染试验

1.1 标准急性试验

用添加 10 % 胎牛血清的 RPMI 1640 作为生长培养基，在 H9 细胞中生长人免疫缺陷病毒 HIV - 1 的高滴度储备毒株（ HTLV - IIIB ）. 经低速离心除去细胞碎片并将上清液贮存于 - 70 ℃下备用. 在一典型试验中，将 C8166 T …淋巴母细胞样细胞与 10TCID$_{50}$ HIV - 1 于 37 ℃一起保温 90 分钟，然后用磷酸盐缓冲盐水（ PBS ）洗三次. 将 2 × 10^5 个细胞的等分样品重新悬浮在加于 6ml 培养管内的

1.5ml 生长培养基中，并立即加入从 0.2 至 200 μ M 对数稀释度的试验化合物。将试验化合物溶解在 70 % 乙醇中并使培养物中乙醇的终浓度小于 1 %。将培养物置于 5 % CO_2 保持温箱中 37 ℃保温 72 小时。从各培养物中取 20 μ l 上清液，使用等同识别所有核心抗原的商品 ELISA（ Coulter Electronics Ltd.,Luton,UK ），并测定 450nm 光密度以检测各培养物的 HIV p24 核心抗原（ Kinchington et al.,1989,Roberts et al.,1990 ）。为了确定 IC_{50} 值，从含<1%乙醇的未经处理的培养物绘制标准曲线。使用 AZT 和 ddc 作为内部对照。以一式两份样品完成试验。

1.2 耗竭培养基试验

在标准试验系统中，收集细胞培养物，均分并在开始试验前约 18 至 24 小时供入新鲜培养基。加入新鲜培养基刺激细胞进入对数生长期。为了研究在耗竭了培养基的条件下细胞达到汇合的影响，在用于标准急性试验前 - 72 、 48 和 24 小时供入新鲜培养基并等分之。

1.3 光曝露试验

将新溶解的试验化合物样品对分成两等份。将其置于月光下或．黑暗处 2 小时，然后进行标准急性试验。

1.4 预保温试验

将靶细胞与有 00 至 0.2 μ M 对数稀释度的试验化合物预保温 18/24 小时，然后感染 HIV - 1 。按标准急性试验中所述个别处理各不同浓度的样品。

2.慢性感染细胞的试验

2.1 标准慢性试验

用培养基将用 HIV - 1rf(h9rf)慢性感染的细胞洗 3 次以除去细胞外病毒，并与试验化合物（200 至 0.2 μ M）一起保温 3 天。然后用如标准急、性感染试验所述的方法，检测 450nm 光密度以确定 p24 抗原量。为了确定 IC_{50} 值从含 1 % 乙醇的未处理的培养物所得数据绘制标准曲线。使用 RO 31 - 8959(Roche 蛋白酶抑制剂）作为内部对照。试验以一式两份方式进行.

2.2 耗竭培养基试验

在标准试验中收获细胞培养物，均分并于试验前约 18 至 24 小时供入新鲜培养基。加入新鲜培养基刺激细胞进入对数生长期。为了研究在耗竭培养基条件下达到汇合之细胞的影响，在用于标准急性试验之前 72 、 48 和 24 小时在细胞培养物中供入新鲜培养基并等分之.

2.3 光曝露试验

将新溶解的试验化合物样品分成两等份．将其置于日光下或暗处 2 小时，然后进行标准慢性试验.

3.毒性试验

为了试验化合物毒性，将 2×10^5 个未感染细胞的等分样品与同样对数稀释度的试验化合物保温 72 小时（参见 1.1 和 2.1 节）．然后用培养基洗细胞并重新悬浮在 200 μ l 含有 C^{14} 蛋白质水解物的生长培养基中． 5 或 20 小时后收获细胞并检测 C^{14} 掺入量．使用未经处理的细胞作为对照.

下列表 1 中验明了所试验的化合物:

表 1：

编号	化合物

F1	核黄素 5'磷酸盐
F2	核黄素
F3	黄素腺嘌呤二核苷酸
F4	光黄素
F5	光色素
F6	核黄素四烟酸
F7	核黄素四丁酸

开始进行与表 2 中提到的各种化合物相关的试验以获得初步结果。表 2 中给出的 $2C_{50}$ 结果是为进一步证实初步结果；它们与至今进行的重复试验不相符合。试验结果示于形成下述附图的曲线图和下示表不至 10 中：

图 1：化合物 F2、F4（第一次抗原试验）和 F5 在 450nm 的抗原光密度（OD）对浓度（μM）曲线。OD 0.371 处的破折线代表 IC_{50}（活性的）。

图 2：化合物 F1 和 F3 在 450nm 处的抗原光密度（OD）对浓度（μM）曲线。OD 0.371 处的破折线代表 IC_{50}（有活性的）。

图 3：化合物 F2、F3、F4（第一次毒性试验）和 F5 的 C^{14} 摄入（dpm）对浓度的毒性曲线。6035dpm 处的破折线代表 CC_{50}（非毒性的）。

图 4：化合物 F1 的 C^{14} 摄入（dpm）对浓度的毒性曲线。6035dpm 处的破折线代表 CC_{50}（非毒性的）。

图 5：化合物 F4（第二次抗原试验）在 450nm 处的抗原光密度（OD）对浓度（μM）的曲线。OD 0.371 处的破折线代表 IC_{50}（有活性的）。

图 6：作为 C^{14} 摄入（dpm）对化合物 F4（第二次毒性试验）之浓度（μ M）的毒性曲线。6035dpm 处的破折线代表 CC_5（非毒性的）。

图 7：化合物 F6 和 F7 在 450nm 处的抗原光密度（OD）对浓度（μ M）的曲线。OD 0.371 处的破折线代表 IC_{50}（有活性的）。

图 8：作为 C^{14} 摄入（dpm）对化合物 F6 和 F7 之浓度（μ M）的毒性曲线。6035dpm 处的破折线代表 CC_{50}（非毒性的）。

图 9：抗原对照（ddc）。

如表中所示，估计试验化合物抗 HIV 急性和慢性感染之细胞的活性。抗病毒（IC_{50}）和毒性（CC_{50}）数据显示如下。在另一实验系列中，在感染前 72、48 和 24 小时加入新鲜培养基细胞培养物中试验各化合物。进行该实验以研究化合物对于活性分裂和静止阶段之细胞的影响。该数据表明，当在静止期时细胞可能对试验化合物更为敏感。还研究了光照对稳定性、预保温靶细胞和抗非洲人 HIV－1 分离物之活性的影响。曝光两小时对化合物的活性没有影响。与靶细胞预保温提高了其活性，并显示其有显著的抗非洲人 HIV－1 分离物的活性。

表 2（图 1－4）－急性感染标准试验（1.1）

化合物编号/试验编号	IC_{50} 图 1 和 2	CC_{50} 图 3 和 4	SI
F1/1	1 至 20	>200	-
F1/2	<0.4	>400	>1000
F1/3	0.1（图 2）	>800（图 4）	>8000
F2	3（图 1）	>200（图 3）	>60

F3	0.8（图 2）	>200（图 3）	>200
F4	1（图 1）	150（图 3）	150
F5	3（图 1）	>200（图 3）	>60

表 3（图 7 和 8）- 急性感染标准试验（1.1）

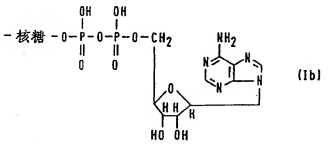
化合物编号/试验编号	IC_{50}	CC_{50}	SI
F7/1	27（图 1）	130（图 8）	5
F7/2	57	>200	>4
F7/3	10	70	7
F7/4	25	140	6

表 4 - 慢性感染标准试验（2.1）

化合物编号/试验编号	IC_{50}	CC_{50}	SI
F7/1	0.2	7	35
F7/2	>20	>20	-
F7/3	10	>200	>20
F7/4	4	75	19
F7/5	26	>200	>7

$-CH_2-(CHOH)_n-Y$

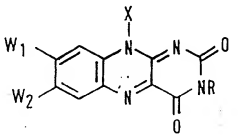
其中 n 是正整数 3 或 4， Y 是 $-CH_2OH$、$-COOH$ 或…COH 或下列通式的基团:



(Ib)

其中 R 是氢或烷基；且其中 W1 和 W2 各自是烷基、羟基、卤素、烷氧基、烷基硫代、硫代或可被取代的芳族或非芳族氮杂环.

6. 权利要求 1 所述的应用，其中黄素或黄素衍生物是有下列通式的化合物:



(Ia)

其中 X 是:

（ i ）氢、核糖醇基、烷基、氢或芳族或非芳族碳环，

（ ii ）下列通式的基团:

表 5 - 急性感染耗竭培养基试验（ 1.2 ）

化合物号	72 小时		48 小时		24 小时	
	IC_{50}	CC_{50}	IC_{50}	CC_{50}	IC_{50}	CC_{50}
F7	10	160	21	100	110	160

表 6 - 慢性感染耗竭培养基试验（ 2.2 ）

化合物号	72 小时		48 小时		24 小时	
	IC_{50}	CC_{50}	IC_{50}	CC_{50}	IC_{50}	CC_{50}
F7	40	75	90	250	60	101

表 7 - 急性感染光照曝露试验

化合物	日光		黑暗	
	IC_{50}	CC_{50}	IC_{50}	CC_{50}
F7	60	>200	60	>200

表 8 - 急性感染预保温试验（ 1.4 ）

感染前靶细胞与试验化合物预保温 24 小时

化合物号	IC50	CC50	SI
F7	5	120	24

表 9（图 5 至 8）－急性感染标准试验（1.1）

化合物编号	IC_{50}	CC_50	SI
F4	13(图 5)	150 (图 6)	12
F6	30-60(图 7)	>200(图 8)	最小 3 － 6

表 10 －急性感染标准试验
用于非洲人 HIV 分离物感染的 C8166 细胞（HTLV 转化的和无限增殖化的 T 淋巴母细胞样细胞）的试验

化合物编号	IC_{50}	CC_{50}	SI
F7	4	150	37.5

表 11（图 10 至 12）－急性感染标准试验（1.1）

化合物编号	IC_{50}	CC_{50}	SI
F7	32	200	6.3
ddc（对照）	0.2		

用化合物 F7 观察到的终点时出现的变化可能是由于靶淋巴母细胞样细胞的性质造成的．即使在同步培养物中也可能有细胞亚群之代谢状况的极细微的改变．这种情况反映在各试验中观察到的成对的抗病毒和毒性值的终点移动上（表 3）．表 5 和表 6 中所列示的结果表明，活性期或静止期的细胞培养物对试验化合物可能有不同的敏感性．

患者治疗

对35名患者进行治疗，对其30人进行了临床随诊。

I)患者的一般状况

随诊的30名患者中有20名患者的一般状况得到改善。这些患者大部分是身体不适、食欲及体重增加方面的改善。两名患者皮疹改善同时皮肤损伤好转，而一名患者在治疗期间没有发生新的皮肤损伤。一名患者阳萎（在治疗开始前已存在三个月）情况改善，而另两名终止了长期持续的感胃。

ii)患者随诊

少数患者来诊所进行不定期随诊：

1. 一名患者复发了仍坚持治疗的脓肿及脓毒性关节炎。

2. 两名患者在第二周治疗期间再发了下呼吸道感染并有一人发展了症状明显的支气管肺炎。反复涂片检查AAFBS持续为阴性。

3. 两名患者有非定位征象的发热，反复血涂片检查疟原虫均为阴性并且在血液培养物上没有显著生长。某中一名患者对羟氨苄霉毒素反应良好并且现在已不发烧。

4. 一名患者在治疗的第三周里发生胃肠炎。

5. 两名患者有口和阴门－阴道念珠菌病，并在使用制霉菌素阴道栓剂和片剂之后很快再发阴门－阴道念珠菌病。

6. 两名患者还报告有生殖器单纯疮疹反复发作。

iii)毒性

多数病例报告在前两周治疗期间发生毒性并且是短暂的。

两名患者在治疗的第一周经历平均四天的瘙痒，并在没作任何对症治疗情况下自然消失。

报告四名患者在前两周治疗期间有中度腹泻，平均持续四天．这是一各处于副作用或 HIV 感染之自然征象之间的难以归因的症状．但基于其在治疗第一周出现的一致性，和其短暂性质而有理由支持其为一种副作用．

一名患者报告有倦睡表现，另一患者报告尿色发暗． MSU 正常．

两名患者报告腹部不适．

iV)实验室结果

在治疗的第二至第三周三名患者有肝脏酶的短暂升高，没有肝病的临床征象．但酶水平已经回复正常．

上述临床试用报告是目前已使用化合物 F7 进行了几周的临床试用的初步结果，其中以胶囊形式（如下文实施例 4 中所述的胶囊）口服给药的化合物 F7 剂量为:

剂量水平 1： 每天每公斤体重 1mg，分两次口服给药

剂量水平 2： 每天每公斤体重 2mg，分两次口服给药

剂量水平 3： 每天每公斤体重 10mg，分两次口服给药

剂量水平 4： 每天每公斤体重 15mg，分两次口服给药

剂量水平 5： 每天每公斤体重 20mg，分两次口服给药

剂量水平 6： 每天每公斤体重 30mg，分两次或三次口服给药

剂量水平 7： 每天每公斤体重 40mg，分两次或三次口服给药

剂量水平 8： 每天每公斤体重 50mg，分两次或三次口服给药

剂量水平 9： 每天每公斤体重 100mg，分两次或三次口服给药

下列具体实施例说明按照本发明配制的组合物:

实施例 1

由下列成分配制组合物:

核黄素－5'－磷酸	10mg
无菌水	2ml

以提供用于治疗病毒感染的每天一次给药的 10mg 单位剂量的核黄素.

实施例 2

由下列成分配制组合物:

核黄素－5'－磷酸	30mg
无菌水	2ml

以提供用于治疗病毒感染的每天一次给药的 30mg 单位剂量的核黄素.

实施例 3

可在 2ml 或 5ml 单位量无菌水中并基于活性成分: 核黄素－5'－磷酸、核黄素、黄素腺嘌呤二核苷酸、光黄素、光色素或其混合物, 制备剂量为: 每毫升 10mg、每毫升 25mg 和每毫升 50ng 的与实施例 1 和 2 中所述者相似的组合物.

实施例 4

配制下列胶囊

规格: 25mg、50mg、100mg、200mg、400mg

类型: 2 部分硬明胶

组合物: 化合物 F7 与微晶纤维素 Ph.Eur 166.4/156.7/118.6/108.7/50mg 混合, 给出胶囊重量为

191.4/206.7/218.6/308.7/450mg 的胶囊.

图.1

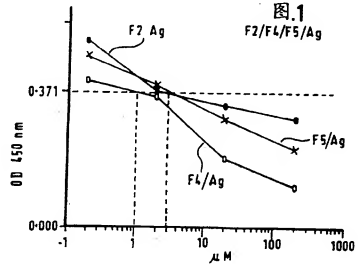


图.2

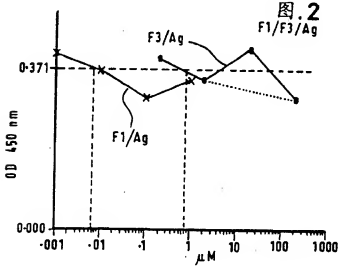


图.3

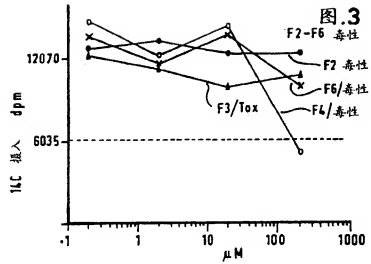


图.4

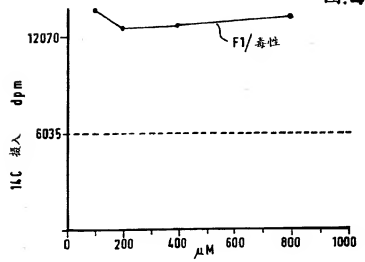


图.5

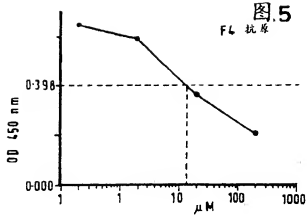


图.6

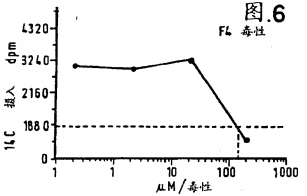


图.7

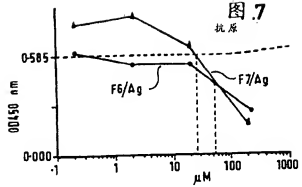


图.8

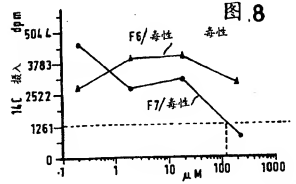
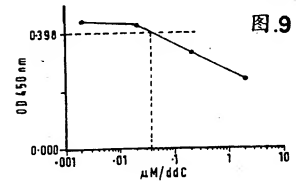
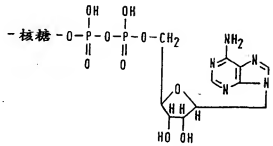


图.9



$-CH_2-(CHOH)_n-Y$

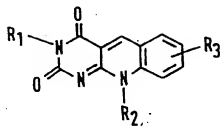
其中 n 是正整数 3 或 4， Y 是 $-CH_2OH$、$-COOH$ 或…CHO 或下列通式的基团：



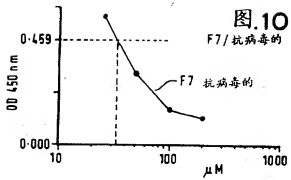
(Ib)

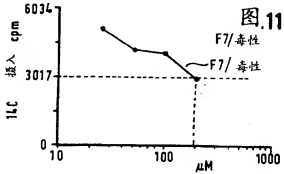
其中 R 是氢或烷基；且其中 W1 和 W2 各自是烷基、羟基、卤素、烷氧基、烷基硫代、硫代或可被取代的芳族或非芳族氮杂环。

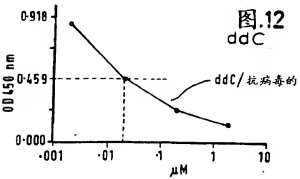
7. 权利要求 1 所述的应用，其中黄素或黄素衍生物是有下列通式的化合物：



(VIII)







其中 R_1 是氢或烷基基团，

R_2 是烷基基团或核糖醇基基团，且

R_3 代表氢或用烷基基团单或二取代的外碳环.

8. 权利要求 1 中所述的应用，其中黄素或黄素衍生物是光色素、玫瑰黄素、羟基黄素、咯嗪或其衍生物、8α－N（3）－组氨酰黄素、8α－N（1）－组氨酰黄素、8α－半胱氨酰黄素硫酯、6α－S－半胱氨酰黄素硫酯、光黄素、5－脱氮杂黄素、核黄素，FMN 或 FAD 的 5－碳酰－5－脱氮杂或 1－碳酰－1－脱氮杂类似物、黄素－1，N^6－亚乙烯基腺嘌呤二核苷酸、9－甲基黄素、9－苯基黄素、9－苄基黄素、9－环己基黄素、6，9－二甲基黄素、6，7，9－三甲基黄素、9－羟乙基黄素、9－二羟丙基黄素、6，8，9－三甲基黄素、乳黄素、黄素－9－羧酸、6，7－二甲基黄素－9－羧酸或聚殖黄素（Schizaflavin）.

9.前列权利要求任何一项所述的应用，用药剂量为每天每公斤体重至少约 10mg .

10.前列权利要求任何一项所述的应用，其中药物是可注射形式的.

11.用于制造可预防或治疗因病毒感染引起之疾病的药物的黄素或黄素衍生物.

12.如权利要求 11 中所述的并如权利要求 2 至 8 的任何一项中限定的黄素或黄素衍生物.

13.用于预防或治疗因病毒感染引起之疾病的药物组合物，其特

征是包含黄素或黄素衍生物.

14.权利要求 13 中所述的组合物,其中黄素或黄素衍生物是如权利要求 2 至 8 的任何一项中限定的.

15.权利要求 12 或 13 中所述的组合物，该组合物包括单位剂量至少约 35mg 的黄素或黄素衍生物连同医药或兽药上可接受的稀释剂，赋形剂或载体.

16.权利要求 15 中所述的组合物，其中单位剂量为大约 35mg 至大约 100mg .

17.权利要求 16 中所述的组合物，其中单位剂量是大约 250 至 500mg .

18.权利要求 15 至地 7 中任何一项所述的组合物,其为可注射形式的.

19.权利要求 18 中所述的组合物，其为在无菌水中的溶液形式的.

20.给药之前容纳药物的容器，所说的容器在给药过程中是可由医务工作人员操作的，并含有从容器排入患者体内或给药装置的黄素或黄素衍生物，且所说的容器带有一使用黄素或黄素衍生物作为预防或治疗因病毒感染引起之疾病的药物的说明书.

21.下述部分的组合:

（ a ）为医药应用而配制的黄素或黄素衍生物，和

（ b ）使用所说的配制的黄素或黄素衍生物制造用于预防或治疗因病毒感染引起之疾病的药物或其应用于所说治疗的说明书.

22.权利要求 21 的组合，其中治疗是说明书中提到的，并且是治疗 HIV 感染.

23.权利要求 22 的组合，其中 HIV 感染是慢性感染。

24.制造用于控制和治疗病毒感染之药物的方法，该方法包括配制抗病毒使用的黄素或黄素衍生物。

25.作为抗病毒剂的黄素或黄素衍生物，连同另一种具有抗病毒活性的化合物，作为在抗病毒治疗中同时、分别或相继使用的联合制剂。

26.预防或治疗因病毒感染引起之疾病的方法，该方法包括给患有这种疾病的患者治疗投用有效量的黄素或黄素衍生物，或给有感染危险的患者预防投用有效量的黄素或黄素衍生物。

27.权利要求 26 中所述的方法，其中给药量是每公斤患者体重至少约 1 至 10mg 或更多。

28.使用不知道有任何医药实用性的黄素或黄素衍生物作为抗病毒剂。

29.用于预防或治疗因病毒感染引起之疾病的黄素或黄素衍生物。

30.如权利要求 9 中所述的并如权利要求 1 - 8 中任何一项所限定的黄素或黄素衍生物。

31.用于治疗至少处于慢性感染阶段之哺乳动物对象的 HIV 感染的抗病毒剂，该抗病毒剂具有细胞靶及可能也有病毒靶，并且是黄素或黄素衍生物，其于细胞内作用于受 HIV 慢性或急性感染之哺乳动物细胞中的细胞代谢，以阻断或抵消病毒感染对病毒感染的无症状期及无症状后阶段之细胞的影响。

32.权利要求 31 中所述的抗病毒剂，其为核黄素衍生物。

33.体外诊断测定方法，该方法包括在以给有 HIV 感染的哺乳动

物患者投用黄素或黄素衍生物以治疗患者后，采集该患者的细胞，并对外部的并从患者体内分离的细胞样品进行试验，以确定病毒感染的活性和/或进程。